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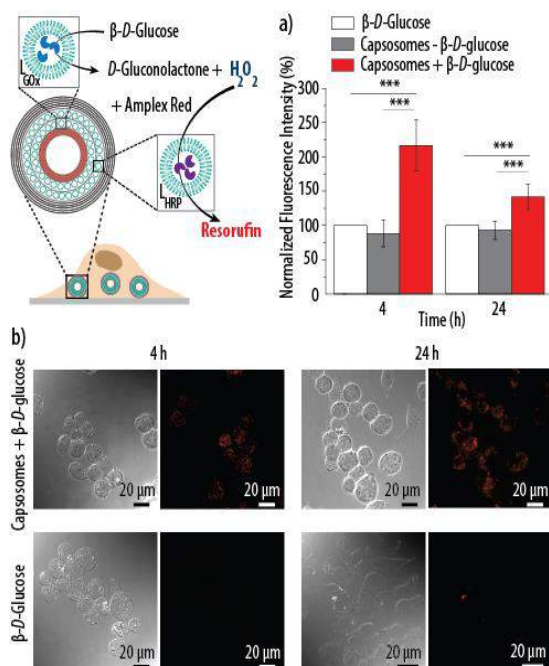
Artificial Organelles: Intracellular Sub-compartmentalized Microreactors to Conduct Enzymatic Cascade Reactions

Maria Godoy-Gallardo¹, Cédric Labay¹, Vasileios D. Trikalitis¹, Paul J. Kempen¹, Jannik B. Larsen¹, Thomas L. Andresen¹, and Leticia Hosta-Rigau^{*1}

1: Department of Micro- and Nanotechnology, Technical University of Denmark, Denmark.

*Corresponding author email: leri@nanotech.dtu.dk.

Cell organelles entrap a set of enzymes to achieve specific reactions within confined sub-compartments. Cell disorders can be treated by replacing malfunctioning organelles by artificial ones. Although several attempts have been made to encapsulate enzymes within carriers, only a few have succeeded employing a multiple-compartment system.¹⁻³ The aim of the present study is to demonstrate that a multistep pathway could be conducted intracellularly by employing a capsosomes which consist of polymer capsules entrapping liposomes as sub-compartments.



Intracellular activity of capsosomes containing enzymeloaded Liposomes. (a) Fluorescence intensity of the enzymatic cascade conversion by fluorescence spectroscopy. (b) Differential interference contrast (DIC) and confocal laser scanning (CLSM) microscopy images.

Glucose oxidase (GOx) and horseradish peroxidase (HRP) were encapsulated within separated liposome compartments of capsosomes in order to conduct a bienzymatic cascade reaction. Briefly, the β -D-glucose substrate is converted into D-gluconolactone and H_2O_2 , which is used by HRP to convert the substrate Amplex Red into the resorufin fluorescent product. In order to perform the enzymatic reaction intracellularly the cell uptake of capsosomes by a macrophage cell line was assessed by flow cytometry and confocal laser scanning microscopy (CLSM). After confirming the successful internalization of the carriers, we verified their functionality by incubating the cells with the internalized capsosomes with β -D-Glucose and Amplex Red for 4 and 24 h. The conversion into the fluorescent resorufin inside the cells was confirmed by fluorescence intensity measurements and by CLSM. Furthermore, capsosomes were able to perform multiple rounds of enzymatic cascade reactions. Therefore, it was demonstrated the capsosomes re-usability and their ability to conduct enzymatic reactions in a continuous and sustained manner, a crucial issue for the creation of successful artificial organelles that are to perform as “cell implants” inside the body.

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